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Physiological factors associated with genetic resistance to fowl typhoid

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NOTE TO USERS

PHYSIOLOGICAL FACTORS ASSOCIATED WITH GENETIC RESISTANCE TO FOWL TYPHOID

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INTRODUCTION

Some individuals within a species are relatively immune to an endemic disease, while other individuals succumb rapidly when exposed. This observation has induced a number of investigators to study the genetic nature of resistance. Their success in establishing genetically resistant and susceptible strains within a number of animal species has recently been extensively reviewed by Cowen (1948). Earlier reviews of this nature were those of Cowen (1933), Lambert (1933), and Hill (1934).

Established strains within a species that differ widely in their genetic resistance provide excellent material for investigating the physiological basis of disease resistance. What defense mechanisms does the recipient of resistant genes possess that are not present in susceptible individuals of the same species? An answer to this question could be of importance in disease prevention.

This investigation was designed specifically to study two aspects of this problem, the relationship of body temperature and phagocytic activity of polymorphonuclear leucocytes to genetic resistance in strains of chickens differing in their resistance to Shigella gallinarum, the etiological agent of fowl typhoid.

REVIEW OF LITERATURE

Fowl typhoid epidemics in domestic fowls have been reported since the eighteenth century. Klein (1889) in describing an epidemic among fowls in England gave the etiological agent of fowl typhoid its first definite name, Bacillus gallinarum. Later investigators have placed the organism in the genus Shigella on the basis of its biochemical reactions or in the genus Salmonella according to its antigenic structure. The name Shigella gallinarum is used in this report.

Knupp and Dearstyne (1925) reported the disease as characterized by an acute intestinal and generalized bacteriemia. Infected birds show an unkempt appearance of the feathers and a sulphur colored fecal discharge. Face, comb and wattles usually are highly anemic. Liver and spleen are enlarged and show areas of necrosis due to bacterial toxins. An early increase in body temperature is evidenced along with a high degree of leucocytosis and a reduction in number of erythrocytes. The leucocytosis is characterized by a consistent increase in the polymorphonuclear leucocytes with a corresponding decrease in lymphocytes.

Since leucocytosis and fever temperature are characteristic responses to Shigella gallinarum, the study of their relationship could be important to an understanding of natural immunity.

The importance of phagocytosis by leucocytes in immunity was established through the classical studies of Metchnikoff (1907). He visualized microphages or polymorphonuclear leucocytes as playing the major role in immunity with macrophages or fixed phagocytic cells being of secondary importance. Later investigators have revealed that immunity in certain diseases may

depend largely on macrophages. The scope of this review is not sufficient to cite the extensive literature comparing the relative importance of the two phagocytic systems. It suffices to state that modern immunology recognizes that both types of phagocytic cells, as well as humoral elements and other factors, are all involved in the body's defense mechanism.

Ingestion of bacteria by phagocytes is only a job half done in wiping out invading organisms. If phagocytes are unable to digest the bacteria, they will transport them to various parts of the body where upon death of the phagocytes, liberated bacteria will initiate new foci of infection. This point is of special interest, since experiments reported here involved phagocytosis and subsequent intracellular digestion. Rous and Jones (1916) demonstrated that living leucocytes protected ingested bacteria from lytic action of antisera and from germicidal power of potassium cyanide. Their experiments were not concerned with the ability of the leucocytes to subsequently digest phagocytosed bacteria.

Mudd et. al. (1933) reported rapid intracellular digestion in vitro of Bacterium typhosum by rabbit leucocytes. After fifteen minutes of ingestion many of the phagocytosed bacteria were swollen, stained faintly or irregularly, and often appeared as mere indistinct shadows which could scarcely be recognized. When percentage of leucocytes containing stainable bacteria was determined after rotating the phagocytic mixture for various times an interesting phenomenon was observed. The phagocytic index increased with time of incubation during the first eight minutes; but surprisingly, longer periods of incubation showed a decrease in percent of leucocytes containing bacteria. Their explanation was that after eight minutes of incubation the rate of intracellular digestion of bacteria was greater than ingestion rate.

In phagocytosis studies using virulent and avirulent strains of Salmonella typhimurium, Pike and Mackenzie (1940) reported rapid destruction of avirulent organisms by an in vivo leucocytic exudate. In order to have sufficient cells for phagocytosis studies, mice were injected intraperitoneally with heat killed staphylococci twenty-four hours previously. Smears made from the leucocytic exudate twenty minutes after injecting virulent strains of the organism revealed abundant phagocytosis. On the other hand, avirulent strains disappeared so rapidly that smears showed only an occasional free or ingested bacillus. Their in vitro studies revealed no significant intracellular digestion after thirty minutes of incubation. Intracellular lysis was demonstrated after several hours of incubation. In parallel tests Eberthella typhosa was found to be more susceptible to intracellular digestion, but lysis was not as rapid as Mudd et. al. (1933) had observed in rabbit leucocytes.

Cottingham and Mills (1943) in vitamin deficiency studies on mice, white rats and guinea pigs concluded that in vivo phagocytosis counts were highly unsatisfactory and variable when compared with in vitro methods. However, results from both methods led to similar conclusions in regard to vitamin deficiencies. After one hour digestion in vitro a majority of the polymorphonuclear leucocytes containing bacteria showed evidence of intracellular lysis. Type I pneumococcus, a coagulase-positive staphylococcus, and Micrococcus candidus were used in their experiments.

The above experiments indicate that polymorphonuclear leucocytes in vivo or in vitro can phagocytose and digest a wide range of bacterial species.

After the classical demonstration by Pasteur et. al. (1878) that the

fowl's immunity to anthrax was dependent on its high body temperature, Wagner (1890) showed that the induced susceptibility was due to a reduction in the phagocytic activity of leucocytes at the low temperature. Since then considerable interest has been shown in the relationship of temperature to phagocytosis.

Ledingham (1908) observed increasing phagocytosis with elevations in temperature from 18° to 42°C. Madsen and Wulff (1919) claimed optimum temperature for phagocytosis in vitro was that of the animal's body at time of removal of the leucocytes. For normal individuals maximum phagocytosis occurred for humans at 37°C., guinea pigs at 39°C., and for fowls at 41°C. Should a fever develop in an individual, according to these authors, maximum phagocytosis would take place at the fever temperature.

Ellingson and Clark (1942) in studies on the effect of artificial fever on resistance observed rapid reduction of antibody titers during induced fever in rabbits. Their phagocytosis studies revealed increasing ingestion of staphylococci by both human and guinea pig leucocytes up to a temperature of 40°C.

In a more extensive study, using guinea pig and rabbit polymorphonuclear leucocytes, Harmon, et al. (1946) observed enhanced phagocytosis of Staphylococcus aureus with increments of 5°C. within a temperature range of 22° to 42°C. When they extended the temperature range to 50°C. with 2°C. increments, phagocytosis increased to a point approximating 43°C. and declined rapidly beyond that point.

It appears evident from the above reports that a high or low body temperature could have a profound effect on the phagocytic activity of leucocytes.

High temperatures alone are enough to destroy some invading pathogenic

organisms. Carpenter et al. (1933) showed that the causative organisms in gonorrhea could be destroyed *in vitro* by temperatures no higher than those induced in artificial fever.

Hutt (1935) observed higher temperatures in White Leghorn chicks, which are more resistant to *Salmonella pullorum*, than he found in chicks of the Rhode Island Red breed. Blood counts revealed a higher total erythrocyte and leucocyte number in the susceptible breed, but White Leghorn chicks possessed a higher percent of lymphocytes.

Scholes and Hutt (1942) concluded that body temperature of chicks is of major importance in genetic resistance to *Salmonella pullorum*. Resistant chicks of the White Leghorn breed brooded at 35°C. consistently maintained a higher body temperature from two to ten days of age than did relatively susceptible Rhode Island Red chicks. When chicks of both breeds were brooded at 28°C. the White Leghorns were better able to maintain and raise their body temperatures than were the Rhode Island Reds. Body temperatures in chicks of both breeds were found to rise fairly rapidly in the first eight days after hatching. Paralleling this rise in temperature was a rise in resistance to *Salmonella pullorum*. Artificially induced hypothermia in Rhode Island Red chicks increased susceptibility to the causative organism. By brooding chicks at 28°C. their body temperatures were lowered by amounts ranging from 0.7° to 1.4°F. below the temperatures of control chicks brooded at 35°C. Chicks thus chilled were consistently more susceptible than were the controls. Susceptibility was also increased by hypothermia induced by injection of sodium amytal. On the other hand, resistance was increased by induced hyperthermia at a brooder temperature of 38°C. Further confirmation of the significance of temperature was provided in the observation that

chicks surviving inoculation consistently maintained a higher body temperature than those chicks which succumbed to the disease. In fact, even among chicks that failed to survive, those with higher temperatures lived longer than those with lower temperatures.

Extensive research on the nature of genetic resistance in chickens has been reported by a group of investigators at Illinois (Quisenberry et al., 1935; Roberts and Card, 1935; Roberts et al., 1939; and Severens et al., 1944). Working with Salmonella pullorum resistant and susceptible strains of chicks, they confirmed Hutt's (1935) observation that resistant chicks possessed higher temperatures and higher percent lymphocytes. Even though their susceptible strain possessed a greater total number of leucocytes, the resistant strain excelled in number of lymphocytes from the eighteenth to the thirty-first day of incubation. During the period of greatest susceptibility, the resistant strain possessed from 50 to 100 percent more lymphocytes. In adult birds, where both strains are normally resistant, the susceptible strain possessed more lymphocytes as well as total leucocytes. Taking all the facts together, they considered differences in body temperature to play an insignificant part in genetic resistance and assigned the major role to differences in lymphocytes. Their conclusions were substantiated by a number of uniquely designed experiments. They found resistance to Salmonella pullorum increased rapidly during the first five days in resistant chicks and during the first ten days in susceptible chicks. Occurring simultaneously was an increase in number of lymphocytes in the blood. This lymphocytic increase was more rapid in resistant chicks, as was also the rise in resistance. A reduction in the number of lymphocytes by X-ray radiation was followed by a decrease in resistance. Splenectomized chicks suffered a re-

duction in lymphocytes as well as in resistance. Sham-operated and splenectomized chicks had the same body temperatures, but differed greatly in resistance. Reduction of resistance by X-ray radiation did not change the body temperature.

Scholes (1942) pointed to the danger of assigning casual factors to reduced resistance of X-ray radiated chicks. He observed that in addition to lowering lymphocytes, X-rays also lowered other leucocytes as well as effected other somatic cells and body temperature.

Schnetzler and Hartsell (1940) found that blood serum of White Leghorn hens possessed greater bactericidal power against Salmonella pullorum than serum from Rhode Island Red hens. If this difference was observed in chicks during the susceptible stage, it might account for observed differences in resistance of the two breeds. However, Severens et al. (1944) found greater bactericidal properties in their susceptible strains than in chicks from resistant strains. Tests were made at one, two and seven days of age. A decrease in bactericidal potency occurred with age in both strains.

In a preliminary report on the nature of genetic resistance in fowl typhoid, Shigella gallinarum, Ball (1947) reported higher body temperature alone was insufficient to account for differences in resistance observed. Susceptible chicks evidenced a significantly higher temperature on the third day after inoculation and maintained it throughout the experiment. In spite of the higher temperature only 15 percent of the susceptible strain survived while 85 percent of the resistant strain survived.

There is increasing evidence on the physiological nature of genetic resistance in other animal species. Irwin and Hughes (1933) found whole blood of rats resistant to typhoid, Salmonella enteritidis, possessed a

greater bactericidal power than blood of susceptible rats. The experiment was so designed that the result obtained could have been caused by either serum components or leucocytes.

Reich and Dunning (1941) observed in six strains of rats a high correlation of number of leucocytes and percent polymorphonuclear neutrophils with survival value of the strain as measured by its duration of life. They suggest possibly some direct relationship between a high total number of neutrophils and long life span.

Gowen and Calhoun (1943) found total leucocyte number and genetic resistance to mouse typhoid, Salmonella typhimurium, highly correlated in six strains of mice. Erythrocyte number and proportions of various types of leucocytes appeared unrelated to genetic resistance. The higher total leucocyte number suggests a greater leucopoietic ability for response to infection.

Oakberg (1946) in a study of the livers and spleens of six strains ranging from high resistance to almost complete susceptibility to mouse typhoid, Salmonella typhimurium, observed increasing ability of macrophages to digest phagocytosed bacteria with increasing genetic resistance. Macrophage number and resistance of tissue cells to bacterial toxins were also highly correlated with genetic resistance. Liver cells of resistant mice were able to carry on normal carbohydrate metabolism in the presence of large amounts of toxin while animals of intermediate and low resistance often showed very little glycogen after onset of morbidity.

The above experiments suggest that fixed and wandering phagocytes play a major role in genetic resistance. Factors, such as body temperature, which influence the relative efficiency of the phagocytes, conceivably,

could cooperate in that leucocytes could be more efficient at higher temperatures. The significance of this interplay of factors for resistance as they affect the chick in the typhoid disease caused by Shigella gallinarum will be presented in the following pages.

MATERIALS AND METHODS

The resistant strains of chicks used were those developed and maintained by the Genetics Department at Iowa State College (Lambert and Knox, 1928 and 1932; Lambert, 1932; Gowen, 1937-47). Close inbreeding over a number of years has firmly fixed the degree of genetic resistance. Three of the resistant strains (A, B, and C) were of the White Leghorn breed, while the fourth resistant strain, RR, was developed from a White Leghorn-Rhode Island Red cross followed by selective breeding for resistance. The

Table 1

Comparative resistance of genetically differentiated strains of chicks to Shigella gallinarum

Strain	Inoculated dose	Total	Survived	Survival %
A	$2x10^5$	745	636	85
B	$2x10^5$	181	172	95
C	$2x10^5$	221	194	88
RR	$2x10^5$	167	145	87
S	$2x10^2$	657	100	15

All differences between resistant strains and S are highly significant.

susceptible strain(S) was of the New Hampshire Red breed and was obtained either as eggs or one day old chicks. Comparative reactions of the strains to virulent Shigella gallinarum are shown in Table 1.

Chicks were inoculated intraperitoneally at ten days of age and deaths were recorded thereafter for twenty-one days. Highest mortality occurred from five to seven days after inoculation. Relatively few deaths were observed the last week of the test period. Table 1 reveals a uniformly high resistance for the four resistant strains. The susceptible chicks are

equally uniform in their reaction to a much smaller inoculum, but in the susceptible direction. In studies reported here resistant chicks were from strain A, unless otherwise designated.

The culture of Shigella gallinarum used was kept in a high state of virulence by frequent reisolation from chicks dying following inoculation. Cultures consisting of Gram-negative short rods, that produced acid but no gas in dextrose and maltose, and neither acid nor gas in sucrose and lactose were considered to be Shigella gallinarum. Serological checks were also made.

Bacterial suspensions for inoculations, as well as for other studies, were obtained from 18 hour growth on veal infusion slants. Bacteria were suspended in physiological salt solution containing sufficient bactopeptone (0.05%) to maintain the number of viable bacteria. Concentration of bacteria was estimated with a Gates nephelometer and appropriate dilutions were made. Actual numbers of viable organisms were determined by poured agar dilution plates.

Chicks were reared in battery brooders in which the temperature was maintained by thermostatic control at about 30°C. A standard chick mash was fed all chicks.

Temperatures of chicks were determined rectally by means of a specially constructed thermo-couple and were recorded to the nearest 0.1 degree C. Unless otherwise shown, temperatures were taken between 9:00 and 12:00 a.m. daily. All temperatures given in this report are for the centigrade scale.

Polymorphonuclear leucocytes for in vitro phagocytosis and intracellular digestion studies were obtained from normal chicks approximately eight weeks of age. A sterile leucocytic exudate was induced in the peritoneal

cavity by injection of 25 ml. of a starch aleuronat paste mixed with an equal volume of tryptose broth. After 18 hours chicks were exsanguinated by bleeding from the heart and the serum recovered was used in the phagocytic system. The peritoneal exudate was suspended in physiological salt solution containing heparin 1:500. The suspension was then centrifuged gently and standardized by means of the Neubauer counting chamber to about 50,000 cells per cmm. Leucocytes were used within three hours after recovering from chicks. A fresh bacterial suspension was made, as described above, for each experiment and was diluted to an estimated 5,000,000 bacteria per ml. Leucocytes and serum were first pipetted into paraffined tubes (73 by 8 mm) and bacteria were added just before the phagocytic test was made. One-tenth ml. of each component was added and the tubes were sealed with paraffined corks. The tubes were immediately placed in a water bath and agitated with a lateral motion (120 reversals of direction per minute) for ten minutes. A sample was then removed and smeared on a slide. For intracellular digestion studies, immediately after sampling a tube it was placed again in the water bath and left undisturbed for one hour in order that digestion of phagocytosed bacteria might take place. A second smear was then made as before. All phagocytosis and digestion studies were made at 41°, except in experiments involving temperature effect. In these experiments additional tubes were run at 36° and 46°. Chicks from resistant and susceptible strains were equally represented in each experiment.

After drying, the slides were stained with Pappenheim's pyronine-methyl green stain. On each smear four samples of 100 polymorphonuclear leucocytes were examined for the presence of phagocytosis. The method of evaluating the phagocytic reaction was that of Hamburger (1912) which in-

volved the determination of percent leucocytes containing bacteria. Digestion slides were counted in the same manner; any ingested bacterium that took enough stain to be recognizable was considered as not digested and the leucocyte containing it would be counted. An estimate of the digestive activity for each chick was obtained from the ratio of number of leucocytes containing bacteria before digestion to the number of leucocytes containing bacteria after digestion. The relative efficiency of this method as compared with other possible methods will be discussed later.

BODY TEMPERATURE AND GENETIC RESISTANCE

In a preliminary study no significant differences were observed in normal body temperature of resistant and susceptible strains (Bell, 1947). Temperatures were recorded between 7:00 and 9:00 p.m. In view of the rather large diurnal variation reported for the fowl (Fronda, 1921; Lamoreux and Hutt, 1939), more extensive experimentation appeared desirable.

Diurnal fluctuations in normal body temperature

In order to establish the diurnal variation in temperature for both resistant and susceptible strains, body temperatures of twelve resistant and six susceptible ten-day-old chicks were taken at various times over a period of two days. Results are shown graphically in Figure 1. Mean temperatures for each strain are given in Table 2.

It is apparent that resistant chicks possessed a significantly higher temperature than susceptible chicks; however, this difference was evidenced only during daylight hours. At night when metabolism was low due to inactivity the temperatures of all chicks were reduced to a common level of about 40.5° . Greatest difference between strains occurred during the morning. Resistant chicks reached their peak temperature by noon while susceptible chicks showed a steady increase in tempera-

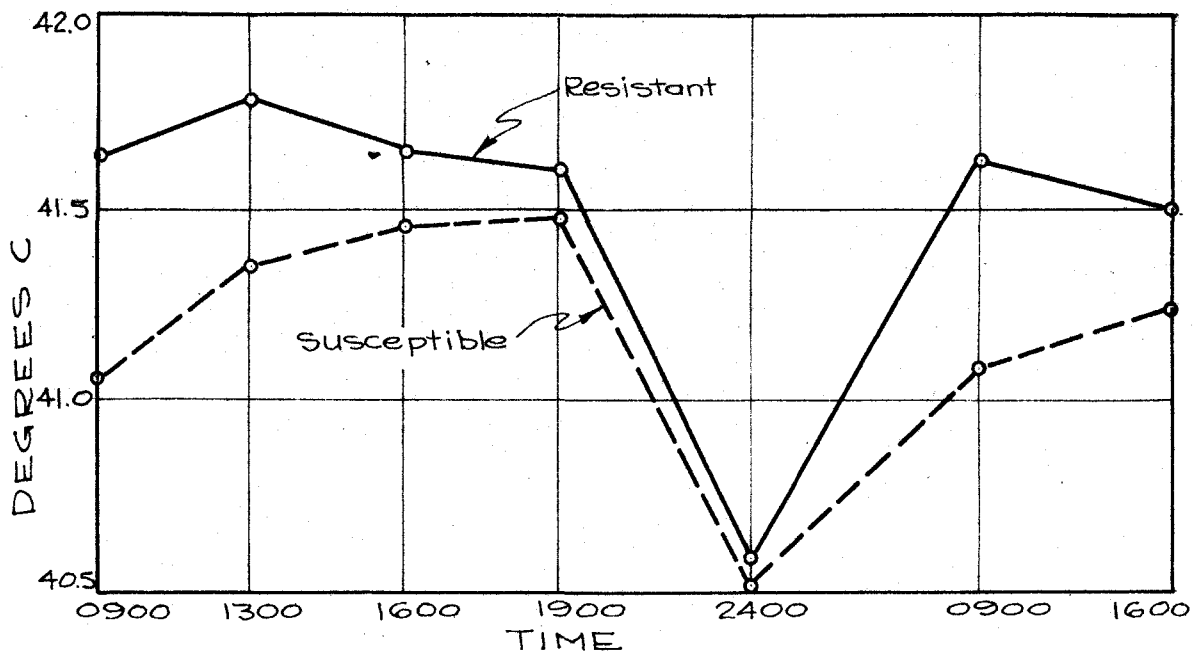


Figure 1. Diurnal fluctuations in normal temperature of resistant and susceptible strains.

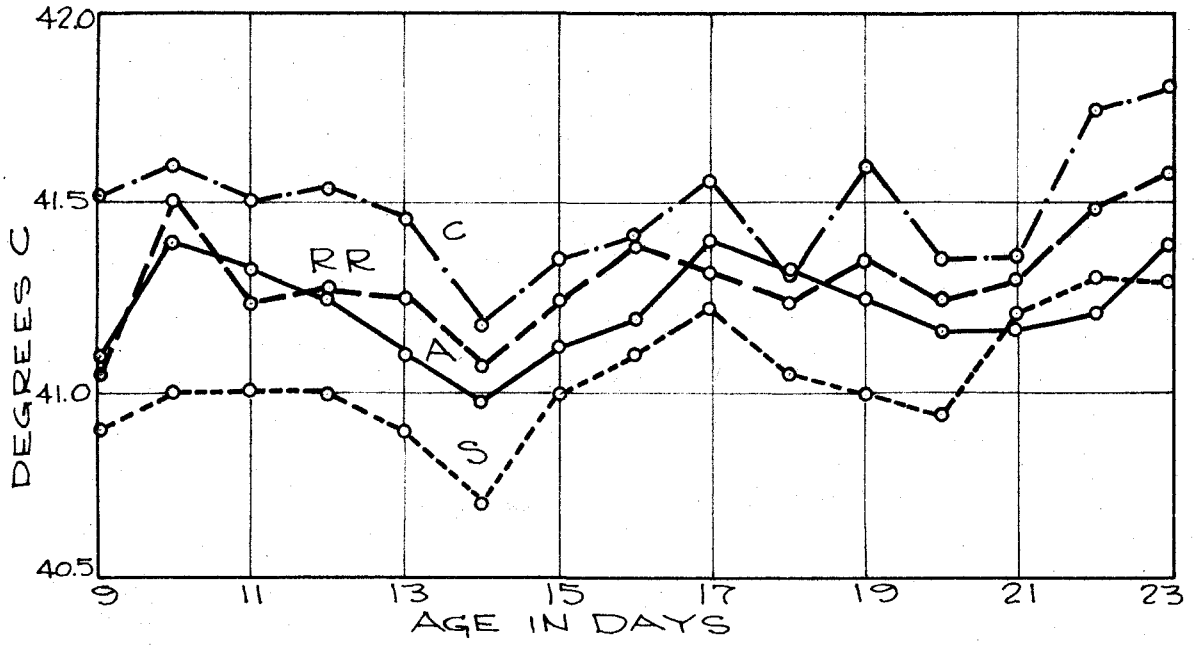


Figure 2. Normal temperatures of strains from nine to twenty-three days of age.

ture until late afternoon. This experiment indicated that a study of temperatures between chicks should be made within a relatively narrow range, preferably between 9:00 a.m. and noon.

Table 2

Diurnal fluctuations in body temperature of untreated resistant and susceptible strains

Time of day	Mean body temperature		Difference
	Resistant	Susceptible	
0900	41.62	41.05	+ .57*
1300	41.79	41.35	+ .44*
1600	41.66	41.45	+ .21
1900	41.62	41.48	+ .14
2400	40.59	40.50	+ .09
0900	41.64	41.08	+ .56**
1600	41.53	41.25	+ .28*
	Average mean difference = 0.33 ± .08**		

* Significant at 5% level.
** Significant at 1% level.

Normal temperatures from nine to twenty-three days of age

To establish the normal temperatures of various strains, daily body temperatures were recorded from nine to twenty-three days of age for 15 chicks of strains A and HR, 10 chicks from C and 13 from the susceptible strain, S. Observations were made daily between 9:30 and 11:00 a.m., thus excluding diurnal variation. Mean daily temperatures for each strain are shown graphically in Figure 2. Average mean temperatures are summarized in Table 3.

Another important influence on body temperature appeared to be variation

between days. The relative importance of these sources of variation can be recognized from the analysis of variance, Table 4. The highly significant mean square due to strains can be attributed to the fact that, in addition to susceptible chicks having a low temperature, resistant strains differed from each other. The breeding program practiced had fixed in each strain

Table 3

Comparative mean normal body temperatures for strains

Strain	Number of chicks	Days observed	Mean body temperature
A	15	15	41.23 ± .024
C	10	15	41.49 ± .019
NR	15	15	41.31 ± .017
S	13	15	41.05 ± .021

The susceptible strain, with two minor exceptions, consistently evidenced a lower temperature than resistant strains.

its own particular mechanism for regulating body temperature. The highly significant mean square due to days was partly due to a slight increase in body temperature with age, but it is readily apparent from Figure 2 that age contribution was small. The major cause of variation between days was inherent in the experimental setup. Chicks were reared in brooders with temperature thermostatically controlled; but, the chicks had access to an unheated portion from which feed and water were obtained. They stayed in this section during most of the daylight hours and daily body temperatures were closely associated with variations in room temperature. Since strain by day interaction was small, strains must have reacted alike to fluctuations in room temperature. Therefore, differences between strains would be only slightly influenced by environmental temperature changes. When total variance for body temperature was separated into its component parts it was found

that independent causes within strains accounted for 66 percent of the total, strain differences 21 percent, day differences 12 percent, and strain by day interaction only 1 percent. Genetic control of 21 percent might seem low until it is compared with other characteristics. Cowan and Calhoun (1943) reported strain differences in mice accounted for 26 percent of variance in total leucocytes and 22 percent erythrocytes. This amount of genetic control was considered by the authors to be rather high.

Table 4

Analysis of body temperatures for period nine to twenty-three days

Source of variation	Degrees of freedom	Mean Square	Components of mean square
Total	794		
Strains	3	5.65**	E + 10.2 I + 197.2 S
Days	14	0.94**	E + 10.2 I + 53 D
Strains X Days	42	0.11	E + 10.2 I
Within	735	0.09	E

** Significant at 1% level

Source of variation	Percent of total variance
Strain effect, S	21
Day effect, D	12
Strain by day interaction, I	1
Independent, E	66

Relation of normal body temperature to resistance

The relative resistance of various strains can now be compared with their normal body temperature. Such a comparison is made in Table 5.

Resistant strains evidenced only slight differences in mortality, but

these differences closely paralleled differences in body temperature. Even though susceptible chicks were inoculated with only 200 bacteria, the association of low temperature and susceptibility is obvious. These data show a correlation of 0.83 between body temperature and fowl typhoid resistance.

Table 5

Relationship of normal body temperature to resistance (survival percent from Table 1 and temperatures from Table 3)

Strain	Survival %	Mean body temperature
C	88	41.5
RR	87	41.3
A	85	41.2
S	15	41.0

In testing the resistance of four strains of White Leghorn chicks not previously described here, an excellent opportunity was provided for further study of the relationship of body temperature to genetic resistance. These strains, designated WA, WB, WC and WG, were more or less distinct and had been selected one year for resistance to fowl typhoid. A moderate amount of inbreeding had been practiced in each strain. Chicks from strains A, B, and S were included in the experiment as controls for both ends of the resistance scale. Normal body temperatures were recorded on ten-day-old chicks previous to inoculation and deaths were observed for 21 days after inoculations. Summarized results are shown in Table 6.

The number of individuals representing strains A and B was small, hence values shown for these strains are less accurate. They are sufficient to establish the relative positions of various strains. Before making strain comparisons it should be noted that the S strain received an inoculum many

thousand times smaller than other strains. The difference in resistance was much greater than indicated by the survival percents. Association of high body temperature with genetic resistance in this experiment gave a significant correlation of 0.94.

Table 6

Normal body temperature at ten days of age and subsequent resistance

Strain	Mean body temperature	Inoculated dose	Inoculated	Survived	Survival %
B	42.02	$1X10^8$	5	4	80
A	41.72	$1X10^8$	4	3	75
WA	41.51	$1X10^8$	16	6	38
WB	41.44	$1X10^8$	64	28	44
WG	41.40	$1X10^8$	27	12	44
WC	41.33	$1X10^8$	28	10	36
S	41.25	$1X10^2$	24	8	33

In these strains high body temperature and genetic resistance are closely correlated. The causal nature of this relation remains to be proven.

Body temperature during the course of fowl typhoid

Association of high normal body temperature with genetic resistance to fowl typhoid has been established. This concurs with reported studies on genetic resistance to Salmonella pullorum (Scholes and Hutt, 1942; Severens et al. 1944). Since genetic resistance is determined by a differential response of strains to the disease organism, it appeared important to study temperature response following inoculation. Twenty-one resistant and eight susceptible chicks were inoculated at ten days of age. Daily temperatures were recorded from nine to twenty-four days. In order to detect deviations from normal, nineteen resistant and eight susceptible control chicks were

Table 7

Body temperatures of resistant and susceptible strains, controls and inoculated--Experiment I

Age of chicks (days)	Controls: Mean body temperature: Resistant	Controls: Mean body temperature: Susceptible	Controls: Difference	Inoculated: Mean body temperature: Resistant	Inoculated: Mean body temperature: Susceptible	Inoculated: Difference
9	41.32± .05	41.22± .06	+.09	41.33 ± .04	41.26 ± .04	+.07
10	41.25 ± .05	40.99 ± .07	+.26**	41.27 ± .07	41.02 ± .07	+.25*
				Inoculation time		
11	41.19 ± .04	41.00 ± .08	+.19*	41.50 ± .08	41.08 ± .06	+.42**
12	41.11 ± .05	40.86 ± .04	+.25**	41.58 ± .08	41.14 ± .13	+.44**
13	41.34 ± .04	41.25 ± .06	+.09	41.63 ± .05	41.22 ± .23	+.41*
14	41.46 ± .10	41.56 ± .11	-.10	41.58 ± .07	41.60 ± .14	-.02
15	41.19 ± .05	41.18 ± .09	+.01	41.45 ± .05	41.74 ± .09	-.29*
16	41.33 ± .07	41.16 ± .16	+.17	41.42 ± .04	41.72 ± .24	-.30*
17	41.27 ± .06	41.10 ± .06	+.17	41.44 ± .06	42.05 ± .11	-.61**
18	41.36 ± .04	41.08 ± .06	+.28**	41.52 ± .06	41.95 ± .14	-.43
19	41.48 ± .05	41.24 ± .08	+.24*	41.68 ± .05	42.20	-.52
20	41.37 ± .04	41.10 ± .06	+.27**	41.44 ± .04	41.90	-.46
21	41.39 ± .05	41.16 ± .05	+.23**	41.42 ± .04	41.50	-.08
22	41.25 ± .03	41.02 ± .07	+.23**	41.52 ± .06		
23	41.41 ± .04	41.18 ± .07	+.23**	41.44 ± .11		
24	41.35 ± .05	41.11 ± .06	+.24**	41.47 ± .04		

Average mean difference = 0.178 ± .03**

* Significant at 5 percent level
** Significant at 1 percent level

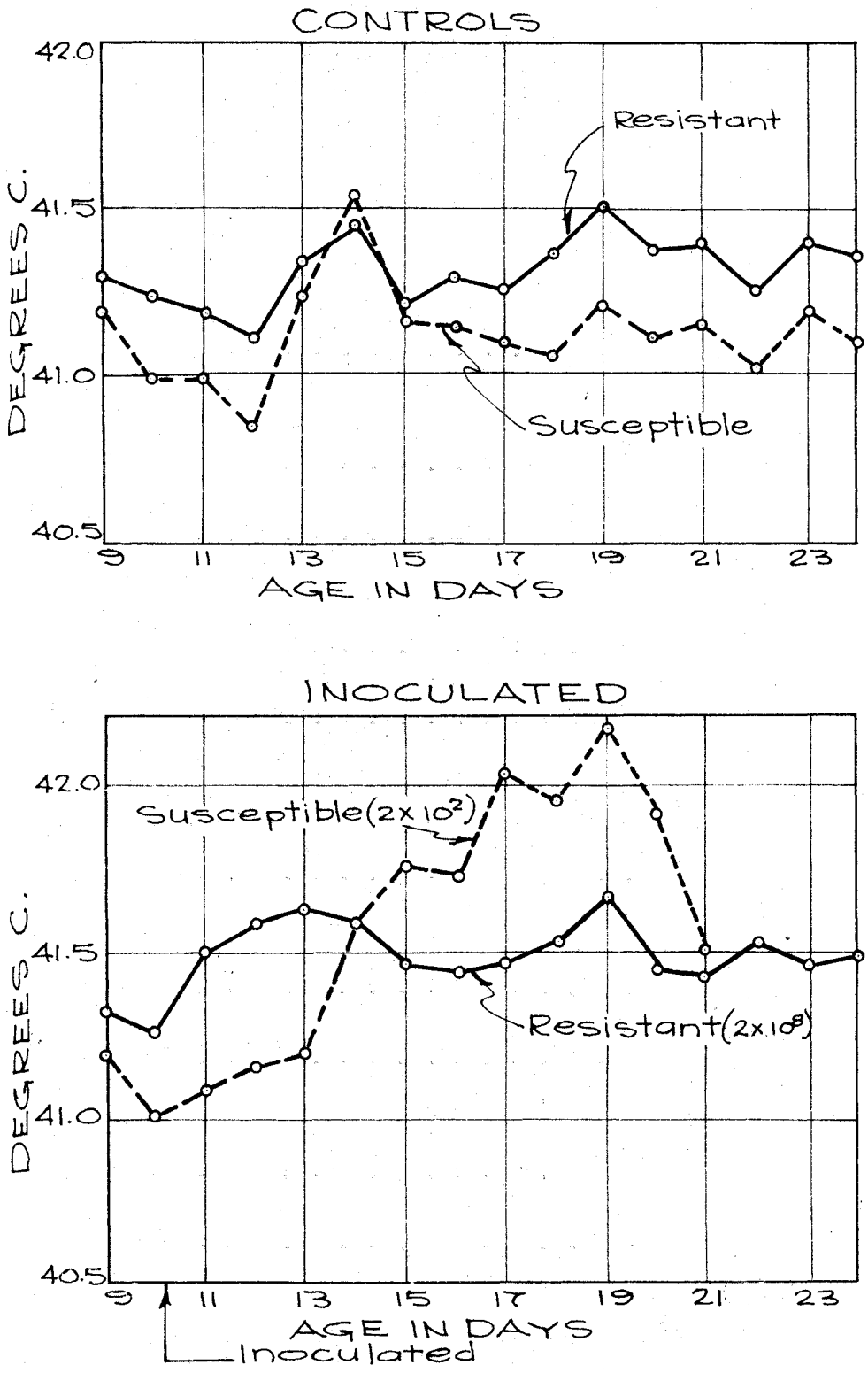


Figure 3. Body temperatures of resistant and susceptible strains - Controls and inoculated, Experiment I

Table 8

Body temperatures of resistant and susceptible strains, controls and inoculated--Experiment II

Age of chicks (days)	Controls: Mean body temperature: Resistant	Controls: Susceptible	Controls: Difference	Inoculated: Mean body temperature: Resistant	Inoculated: Susceptible	Inoculated: Difference
9	41.05 ± .06	41.02 ± .04	+.03	40.99 ± .08	40.99 ± .04	0
10	41.04 ± .05	40.90 ± .05	+.14	41.03 ± .04	40.85 ± .03	+.18**
				Inoculation Time		
11	40.90 ± .05	40.68 ± .08	+.22*	41.15 ± .06	40.83 ± .08	+.22*
12	41.14 ± .06	40.87 ± .09	+.27**	41.14 ± .08	40.91 ± .06	+.23*
13	41.04 ± .08	40.94 ± .06	+.10	41.07 ± .06	41.05 ± .06	+.02
14	41.15 ± .11	41.03 ± .06	+.12	41.27 ± .07	41.44 ± .09	-.17
15	41.18 ± .06	41.03 ± .07	+.15	41.25 ± .06	41.51 ± .13	-.26
16	41.21 ± .04	41.15 ± .05	+.06	41.39 ± .05	41.62 ± .11	-.23
17	41.01 ± .06	40.83 ± .05	+.18*	41.17 ± .05	41.15 ± .14	+.02
18	41.05 ± .07	40.85 ± .04	+.20*	41.25 ± .08	41.15 ± .17	+.10
19	41.08 ± .04	40.89 ± .03	+.19**	41.20 ± .06	41.41 ± .16	-.21
20	41.10 ± .03	40.97 ± .05	+.13*	41.19 ± .05	41.70 ± .20	-.51**
21	41.09 ± .03	40.96 ± .03	+.13**	41.33 ± .06	41.43 ± .21	-.10
22	41.26 ± .05	41.25 ± .05	+.01	41.30 ± .05	41.30 ± .16	0
23	41.15 ± .08	41.31 ± .12	-.16	41.63 ± .09	41.23 ± .14	+.40*
24	41.28 ± .05	41.06 ± .04	+.22**	41.47 ± .10	41.35 ± .16	+.12

Average mean difference=0.12 ± .03**

* Significant at 5 percent level
** Significant at 1 percent level

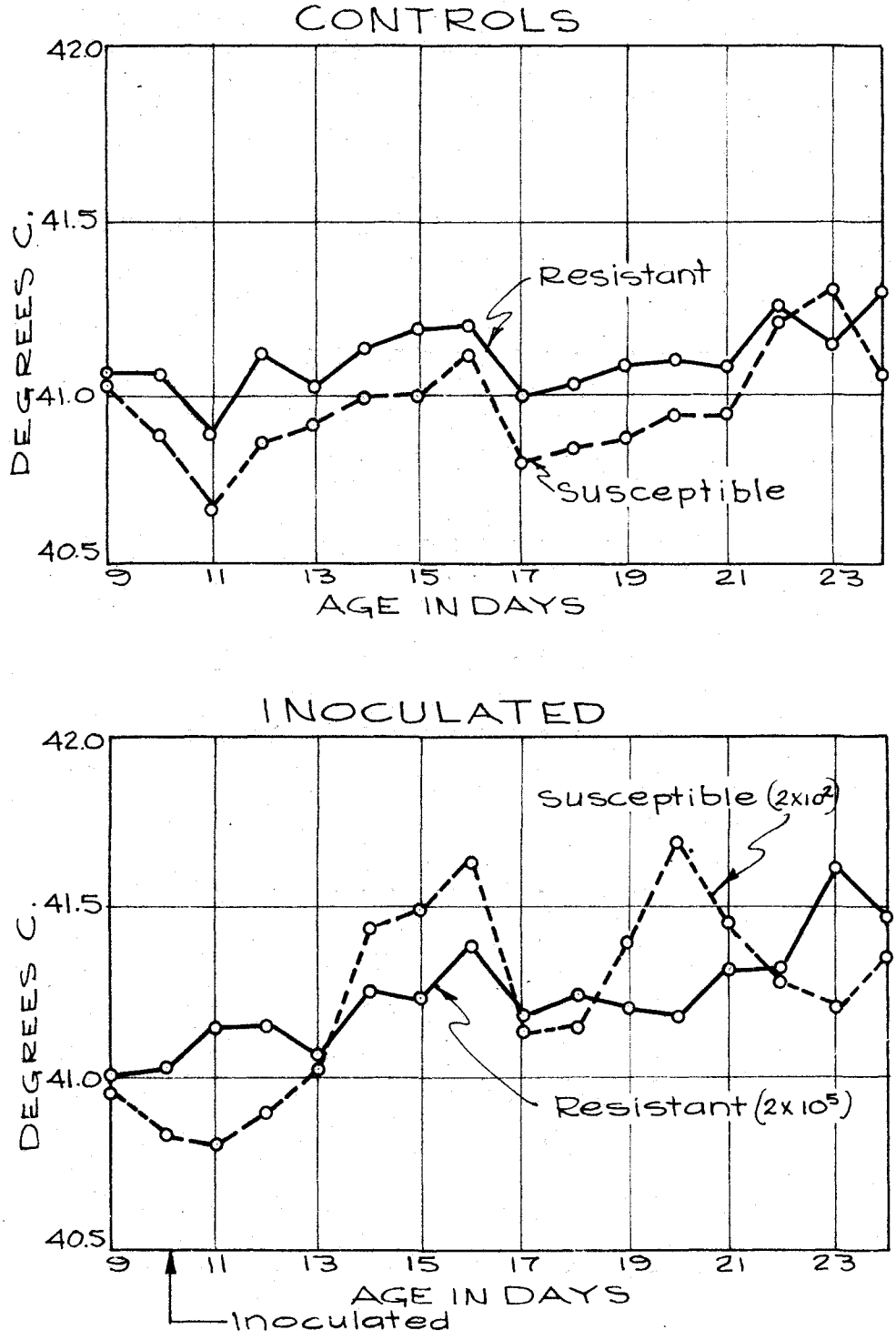


Figure 4. Body temperatures of resistant and susceptible strains - Controls and inoculated, Experiment II

included in the experiment. Results are shown in Table 7 and Figure 3. Higher normal body temperatures in resistant chicks as shown in control comparisons were again evidenced. Most of the daily mean differences were highly significant and the average daily mean difference of 0.18° was highly significant. Results from inoculated chicks were more surprising. For three days following inoculation resistant chicks maintained significantly higher temperatures, but by the fifth day this relationship was reversed with susceptible chicks exhibiting a much higher temperature until all had died by the eleventh day. Within the resistant strain 90 percent survived the test.

The above experiment was repeated using more nearly equal subclass numbers. Fifteen chicks were inoculated in each strain with thirteen resistant and ten susceptible controls. Results shown in Table 8 and Figure 4 are similar to those of the previous experiment. Mortality data for the two experiments are shown in Table 9.

Table 9

Mortality data on resistant and susceptible strains in temperature experiments I and II

Experiment	Strain	Dosage	Inoculated	Survived	Survival %
I	Resistant	$2X10^8$	21	19	90
	Susceptible	$2X10^2$	8	0	0
II	Resistant	$2X10^5$	15	15	100
	Susceptible	$2X10^2$	15	6	40

Differences in both experiments are highly significant

Fever temperature during disease is evidently not a good criterion of genetic resistance in fowl typhoid. These results are not in accord with the findings of Scholes and Hutt (1942). They concluded that chicks which develop a fever temperature following inoculation with Salmonella pullorum

are more resistant to that organism than are chicks which do not. Results of experiments reported here indicate that fever temperature developed during fowl typhoid infection is correlated with susceptibility instead of resistance. Severity of the infection determines the extent of the fever temperature. Fever in susceptible chicks indicates that the battle between host and invader during the incubation period has been lost. Toxins produced by the overwhelming pathogen create disturbances in the circulatory, respiratory and heat regulating mechanisms which result in the observed fever. This does not imply that body temperature is unimportant in disease resistance. Resistant chicks during the first three days following inoculation, possessed significantly higher temperatures. This is the period when the bacteria are relatively few in number and high body temperature could materially aid other defense mechanisms.

Within a strain, chicks that survive can be considered more resistant than those dying. Of the inoculated susceptible chicks in Table 8, six survived and nine died. Daily mean body temperatures for these two groups are illustrated in Figure 5. The trend is similar to that observed between resistant and susceptible strains (Figures 3 and 4). Resistant survivors had higher temperatures immediately following inoculation, but those subsequently dying developed significantly higher temperatures on the fourth, fifth, and sixth days. No significant differences were evidenced between the two groups before inoculation.

Temperature changes immediately following inoculation

Higher temperatures in the resistant strain were noted immediately following inoculation. To investigate this factor further, a within strain

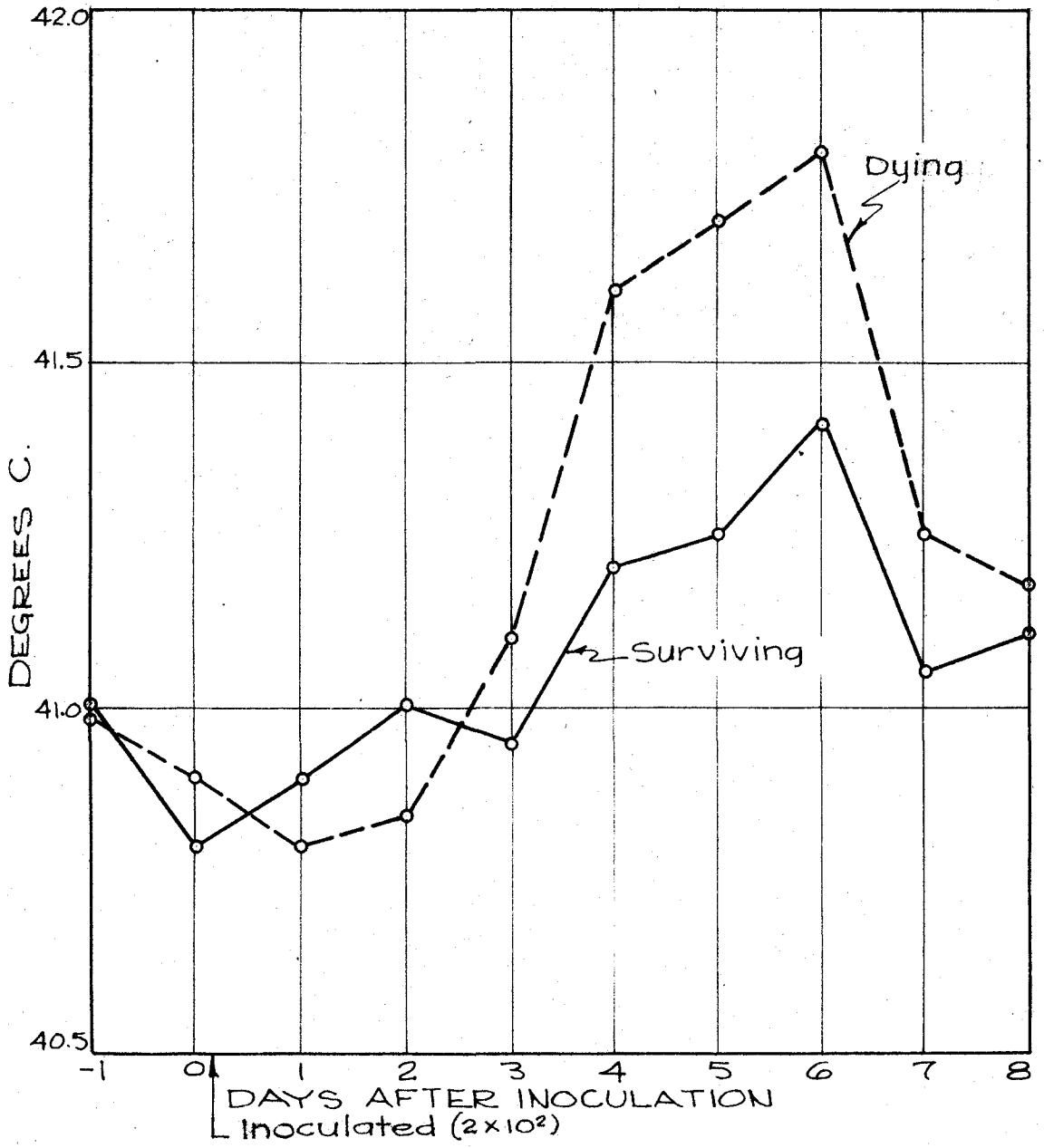


Figure 5. Body temperatures of chicks that subsequently survived or died within susceptible strain.

comparison was decided upon. One handicap to this approach was obtaining sufficient mortality within the resistant strain. On the other hand, it was difficult to obtain sufficient survivors within the susceptible strain. Results shown in Table 10 and Figure 6 are typical for the resistant strain.

Table 10

Body temperatures of surviving and dying resistant chicks immediately following inoculation

Time of day	Mean body temperature		
	Chicks surviving	Chicks dying	Difference
0900	41.66	41.66	0.
Inoculated			
1300	42.17	42.06	+0.11
1600	42.21	41.78	+0.43
1900	41.71	41.11	+0.60*
2400	40.67	40.46	+0.21
0900	41.60	41.32	+0.28
1600	41.67	41.00	+0.67*

Average mean difference $0.38 \pm .09$**

* Significant at 5 percent level
** Significant at 1 percent level

Ten chicks survived and five succumbed to an inoculum of 200 million bacteria. Temperatures were recorded before inoculation and at various time intervals immediately following.

No difference in temperature was observed before inoculation between chicks destined to survive and those later dying. Immediately after inoculation a rapid differentiation occurred. Chicks that survived possessed higher temperatures at each time observed. Only two of the differences were significant, but all were in the same direction. The average mean difference of 0.38^{0} for times observed after inoculation was highly significant. No chicks died or evidenced disease symptoms during the observation period. Mortality did not begin until three days after inoculation.

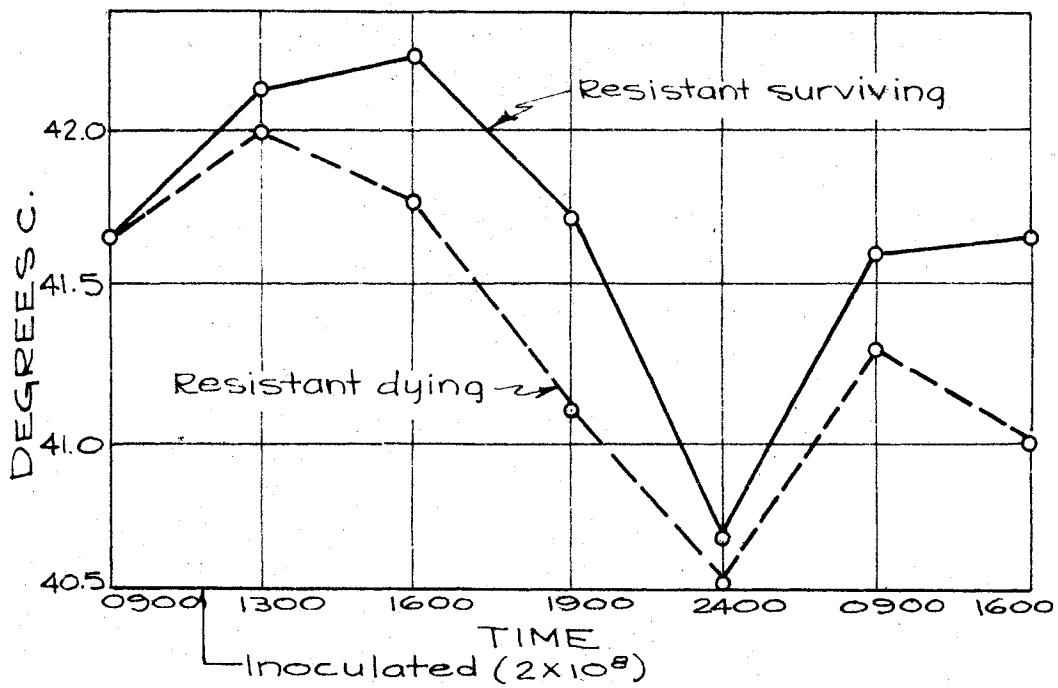


Figure 6. Initial temperature response to inoculation within resistant strain.

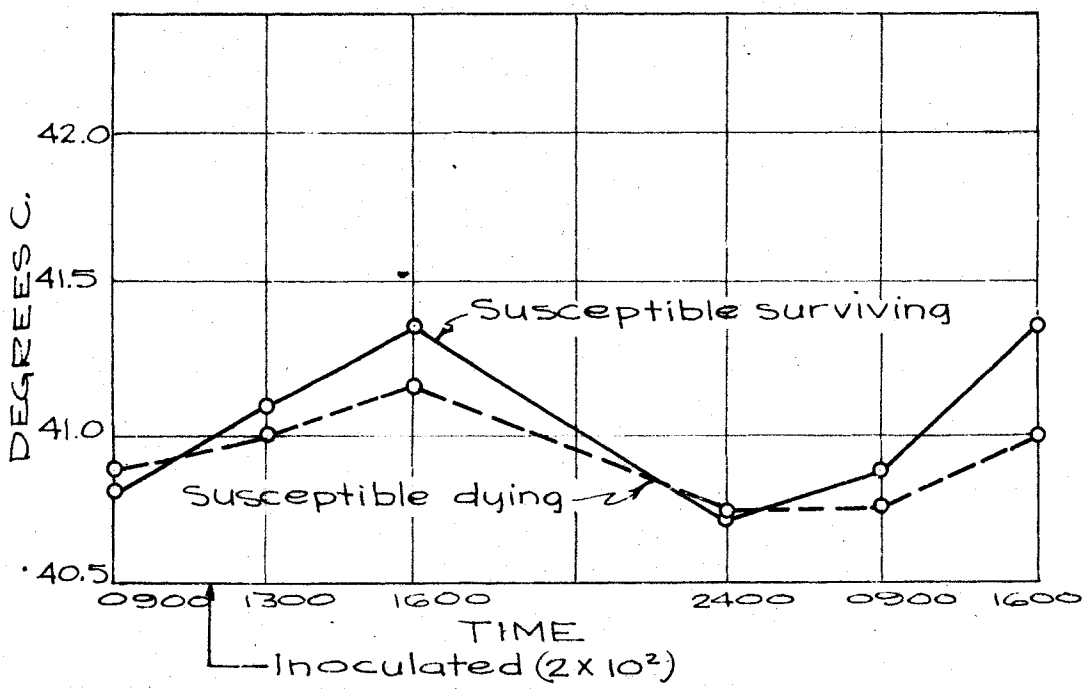


Figure 7. Initial temperature response to inoculation within susceptible strain.

Within the susceptible strain similar results were obtained. Temperature observations were made as described for the resistant strain, but the inoculum contained only 200 bacteria. Six chicks survived and nine subsequently died. The data presented in Table 11 and Figure 7 are typical for the susceptible strain.

Table 11

Body temperatures of surviving and dying susceptible chicks immediately following inoculation

Time of day	Mean body temperature: Chicks surviving	Chicks dying	Difference
0900 Inoculated	40.82	40.88	-0.06
1300	41.10	41.03	+0.07
1600	41.37	41.22	+0.15
2400	40.72	40.73	-0.01
0900	40.88	40.80	+0.08
1600	41.37	41.04	+0.33*

Average mean difference = 0.12 ± .05, t = 2.31 (t at 5% level = 2.78)

*Significant at 5 percent level.

Chicks subsequently dying had a slightly higher normal temperature, but after inoculation survivors consistently maintained the higher temperature. Differences were not as large as those found in the resistant strain, but it should be recalled that the inoculum was many times smaller. Any initial response to such a small inoculum was surprising.

Observations made on strains WA, WB, WC, WG and S provided additional information on body temperature of chicks that subsequently survive or die following inoculation. Results of temperatures taken before inoculation are summarized in Table 12.

Within each strain subsequent survivors possessed a higher normal temperature. The average mean difference of 0.13° was highly significant. For

susceptible strain S the small difference observed agrees with previous experiments. Any resistance present in this strain does not appear to be associated with a higher normal body temperature. Since the W strains possessed considerable heterozygosity for resistance, the relatively large

Table 12

Normal body temperatures of chicks that subsequently survived or died

Strain	Number survived	Number died	Mean body temperature Survived	Died	Difference
WA	6	10	41.62 ± .06	41.45 ± .10	+.17 ± .08
WB	28	36	41.51 ± .04	41.38 ± .08	+.13 ± .09
WC	10	18	41.44 ± .09	41.27 ± .10	+.17 ± .15
WD	12	15	41.44 ± .11	41.37 ± .10	+.07 ± .27
S	8	16	41.31 ± .12	41.22 ± .08	+.09 ± .14

Average mean difference = 0.13 ± .02**

** Significant at 1 percent level

differences in normal temperature observed between subsequent survivors and those that died could have a significant meaning. It suggests that along with the segregation of genes for a higher body temperature there was also combined a greater resistance. Selection for high body temperature in such a population should bring about a corresponding increase in resistance. This statement is speculative, since little is known concerning the complex mechanisms regulating body temperature.

Temperatures of W strains were recorded again four hours after inoculation and are summarized in Table 13. As in previous experiments, survivors in all strains possessed higher temperatures than chicks destined to die. The average mean difference of 0.20° was significant.

Detecting this differential response in different birds immediately following inoculation could be of practical importance in many types of in-

vestigation. In histological studies, tissues could be chosen on the basis of the individual's temperature response during the incubation period of the disease. Thus, tissues from chicks destined to survive or die could be

Table 13

Temperature of chicks subsequently surviving or dying, taken four hours after inoculation

Strain	Number survived	Number died	Mean body temperature Survived	Died	Difference
WA	6	10	41.95 ± .10	41.60 ± .15	+.35 ± .13*
WB	28	36	41.71 ± .05	41.49 ± .12	+.22 ± .15
WC	10	18	41.83 ± .08	41.74 ± .07	+.09 ± .11
WG	12	15	41.72 ± .06	41.59 ± .11	+.13 ± .24

Average mean difference=0.13 ± .02**

* Significant at 5 percent level
** Significant at 1 percent level

studied at any time following inoculation. Any differential response could also aid the geneticist in establishing more efficient selection indexes.

Effect on resistance of artificially reduced body temperature

Another method of testing the correlation between genetic resistance and body temperature is offered when the body temperature is reduced by lowering brooder temperature. Chicks of resistant and susceptible strains were brooded at 30° and 24° following inoculation. Body temperatures were recorded daily for eleven days. The data of Table 14 and Figure 8 indicate that a significant decrease in body temperature was produced in chicks brooded at 24°. Both resistant and susceptible chicks reared at the lower temperature possessed significantly lower body temperatures than their corresponding controls. The average difference between resistant groups was 0.24° and between the susceptible groups a larger difference of 0.47°

Table 14

Temperatures of inoculated resistant and susceptible strains brooded at 30° and 24°

Days after inoculation	Resistant Strain: Mean body temperature: Brooded at 30°	Brooded at 24°	Difference	Susceptible Strain: Mean body temperature: Brooded at 30°	Brooded at 24°	Difference
0	41.72	41.48	+.24	41.45	41.13	+.32
1	41.58	40.92	+.66	41.11	40.63	+.47
2	41.90	41.52	+.38	41.25	40.90	+.35
3	41.95	41.75	+.20	41.38	41.04	+.34
4	42.02	41.72	+.30	41.75	41.32	+.43
5	41.45	41.50	-.04	41.52	41.16	+.36
6	41.60	41.60	0	41.72	41.14	+.58
7	41.68	41.60	+.08	41.79	41.25	+.54
8	41.58	41.43	+.15	41.97	41.30	+.67
9	41.65	41.35	+.30	41.60	41.10	+.50
10	41.78	41.65	+.13	41.48	41.13	+.35
11	41.70	41.20	+.50	41.58	40.80	+.78
Average mean difference = 0.24 ± .06**				Average mean difference = 0.47 ± .04**		

** Significant at 1 percent level

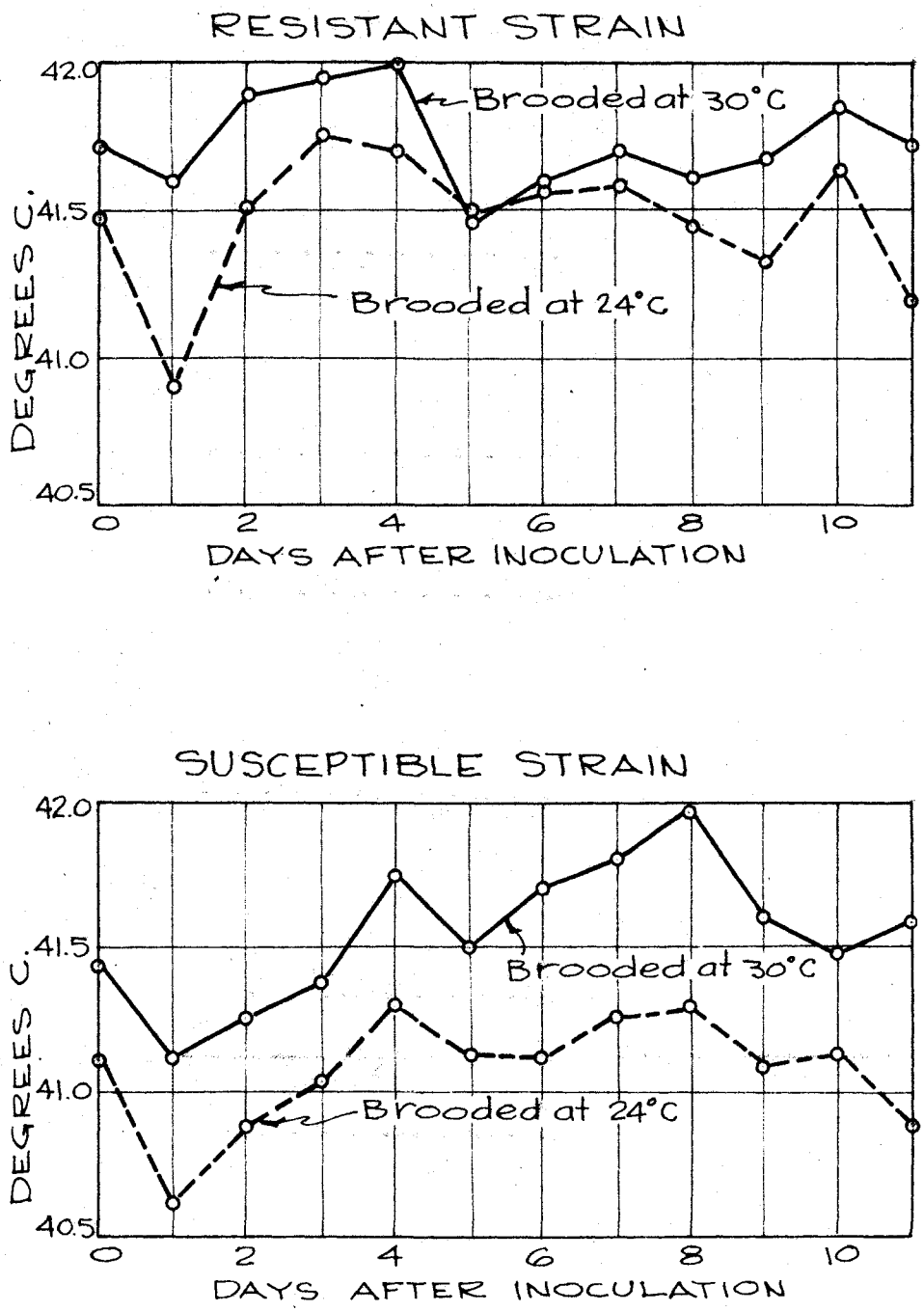


Figure 8. Influence of environmental temperature on body temperature in resistant and susceptible strains.

was evidenced. The temperature regulating mechanism of resistant chicks was more efficient. When exposed to a low environmental temperature, susceptible chicks have nearly twice the internal heat loss of their resistant associates.

Mortality data (Table 15) show that both resistant and susceptible strains were lower in resistance to Shigella gallinarum during hypothermia. Differences were not significant; however, similar trends were evidenced in both strains. Among chicks dying, days to death gives a measure of relative resistance. Chicks dying within the high temperature group of the susceptible strain lived significantly longer than those dying at the lower temperature.

Results from three additional experiments on effect of low environmental temperatures are summarized in Table 16. In principle they agree with the previous described experiment. The third experiment was not well adapted for the test desired as the mortality was so severe that only one chick survived at either temperature. With this exception, mortality was less in every case at the higher temperature. Heterogenity of variances in mortality data prevented combining experiments for a more adequate test of significance. All significant differences in mean days to death were in favor of chicks reared at 30°. These facts indicate that body temperature can have a real effect on resistance to fowl typhoid. The data are likewise concordant with those of Scholes and Hutt (1942) on the effect of reduced body temperature on resistance to Salmonella pullorum.

If these data appear contradictory to results shown in Tables 7 and 8, it should be recalled that fever temperatures in susceptible chicks occurred three to four days after inoculation. By then infection has spread

Table 15

Comparative resistance of inoculated chicks brooded at different temperatures

Strain	Brooder temperature	Inoculated dose	Number inoculated	Number survived	Survival %	Mean days to death
Susceptible	30°	$1x10^2$	11	5	46	$8.3 \pm .8$
Susceptible	24°	$1x10^2$	13	3	23	$5.1 \pm .4$
Resistant	30°	$1x10^8$	5	4	80	4*
Resistant	24°	$1x10^8$	5	2	40	7.3 ± 2.2

* Based on only one individual.

Difference between $\bar{x}$ days to death 8.3-5.1 is highly significant.

Table 16

Effect of low environmental temperatures on resistance

Expt.	Strain	Inoc dose	Brooded at 30°			Brooded at 24°		
			No. of chicks	Survival %	Mean days to death	No. of chicks	Survival %	Mean days to death
II	S	$1x10^2$	20	20.0	9.1 ± .96	20	10.0	6.6 ± .31
III	S	$1x10^3$	13	0	7.3 ± .56	11	9.0	5.8 ± .25
IV	R	$1x10^8$	28	60.7	6.7±1.17	28	32.1	4.7± .30
	S	$1x10^3$*	20	60.0	8.8±1.56	17	47.1	10.1±1.94

Difference in mean days to death between 30°C. - 24°C. -
Experiment II - significant at 5 percent level.
Experiment III - significant at 1 percent level.
Experiment IV - Resistant-significant at 5 percent level.

* Less virulent culture used to increase survival of S strain

throughout the body and the fever is probably the result of toxic damage to the temperature regulating mechanism. The data suggest that a fever temperature during this period is closely associated with susceptibility for both between strains as well as within strain variations.

HUMORAL AND CELLULAR FACTORS IN GENETIC RESISTANCE

High body temperature *per se* is insufficient to account for genetic resistance to fowl typhoid. Within a range of 0.4° internal body temperature is an inherited characteristic. If it is to be a factor in genetic resistance, it must exert its influence through other defense mechanisms during the incubation period of the disease. Phagocytosis is a defense mechanism influenced by temperature. Data on the relative phagocytic ability of polymorphonuclear leucocytes from resistant and susceptible strains are found in Table 20. These results could be confounded with any bactericidins present in the serum. Tests for bactericidins were first made on whole blood and on serum from resistant and susceptible strains.

Bactericidal properties of whole blood and serum against Shigella gallinarum

Since leucocytes and sera for phagocytosis were obtained from chicks about eight weeks old, tests for bactericidins were made at that age. Blood was taken aseptically from the heart and collected in sterile tubes containing sufficient quantity of heparin to prevent coagulation. Bactericidal tests were conducted as follows: A uniform quantity (0.5 ml.) of whole blood and 0.1 ml. of a bacterial suspension diluted to an estimated 5,000 bacteria per ml. were placed in sterile agglutination tubes. The number of viable bacteria in the suspension was checked by poured dilution agar plates.

Tubes were incubated at 41° for two hours. Contents of each tube were then mixed with ten ml. of nutrient agar and poured into petri dishes. Bacterial colonies were counted after 48 hours incubation. All tests were made in duplicate. Typical results are shown in Table 17.

Table 17

Bactericidal action of whole blood of various strains on Shigella gallinarum

Experiment	Number of individuals per strain	Expected colonies per plate	Colonies per plate A	C	RR	S
I	10	500	402	420	534	496
II	10	500	429	383	---	322

Considerable variation was found between individuals within a strain. Differences between strains were not significant. Similar results were obtained with serum. These results indicate that genetic resistance to fowl typhoid is not dependent upon normal bactericidins in whole blood or serum.

Relative resistance to Shigella gallinarum of strains at eight weeks of age

For adequate interpretation of phagocytosis results the relative resistance of strains had to be determined at eight weeks of age. Severens et al. (1944) reported that S. pullorum resistant and susceptible strains were equally resistant by ten days of age. Results in Table 18 indicate that the differences between strains in resistance to Shigella gallinarum at ten days of age is still present in eight weeks old birds of the same strains.

Similar results were obtained with adult birds. There is some increase in resistance to fowl typhoid with age, but the increase is more evident in

the resistant strains than in the susceptible strain.

Table 18

Comparative resistance of strains to Shigella gallinarum at eight weeks of age

Strain	Inoculated dose	Inoculated	Survived	Survival %
A	$2x10^5$	27	27	100
B	$2x10^5$	7	7	100
C	$2x10^5$	4	4	100
S	$2x10^5$	17	1	6

Phagocytosis by polymorphonuclear leucocytes at 41° C.

Normal temperature of the fowl is about 41°. This temperature was chosen for in vitro phagocytosis studies. Adjustment for the difference in body temperatures of resistant and susceptible strains did not seem practical for the in vitro experiments. Leucocytes and sera were obtained from normal individuals. Each sample of serum was tested for the presence of Shigella gallinarum antibodies. The phagocytic mixture of leucocytes, serum and bacteria were incubated as described. Six experiments were conducted with resistant and susceptible strains equally represented within each experiment. A total of twenty individuals were tested. After smears were stained four samples of 100 polymorphs from each individual were examined for the presence of ingested bacteria. Mean percent polymorphs containing phagocytosed bacteria was 9.5 percent for the resistant and 9.2 percent for the susceptible strain. At a constant temperature of 41°, resistant and susceptible strains possessed equal phagocytic abilities. The experiments as conducted allow for any possible differences in normal opsonins. Leucocytes

and serum in each test were from the same individual.

Temperature effects on phagocytosis

Experiments measuring body temperatures following inoculation showed that resistant chicks maintained significantly higher body temperatures during the important incubation period of fowl typhoid.

Phagocytosis *in vivo* of resistant chicks must be occurring at a higher temperature than in the susceptible strain. To determine effect of temperature on phagocytosis, experiments were conducted at temperatures ranging from 36° to 46° with intervals of 5°. Even though the extreme temperatures would not be found *in vivo*, they provided points from which a trend passing through the normal temperature can be established. Polymorphs from most of the chicks in the experiment were tested at all three temperatures. Thus, results shown in Table 19 are not confounded with differences between individuals.

With each increase in temperature there was a corresponding increase in the phagocytic ability of polymorphs. This increase was evidenced within all susceptible individuals and within all resistant chicks, except one. Differences between strains at various temperatures were not significant. For computing regressions of percent phagocytosis on temperature of incubation, the percentage data were transformed into angles (Snedecor, 1946):

$$\text{angle} = \text{arc sin}\sqrt{\text{percent phagocytosis}}$$

The transformation was necessary for tests of significance since the percent values were small and would not be normally distributed. Corresponding angles should approach a normal distribution.

Regressions of angles corresponding to percent phagocytosis on temperature are presented in Figure 9. Highly significant regression coefficients were evidenced in both strains. Greater phagocytic ability resulted with

Table 19

Effect of temperature on *in vitro* phagocytosis

Chicks by strain	Percent phagocytosis 36°C.	41°C.	46°C.
Resistant			
R2378	2.8	8.5	12.5
R2379	---	7.0	16.5
R2390	3.2	5.2	6.5
R2438	3.2	11.5	6.0
R2439	---	6.0	9.8
Average	3.1	7.6	10.0
Susceptible			
S3892	---	10.5	16.5
S3902	---	12.5	19.5
S3903	2.2	4.8	12.0
S3931	2.8	8.5	13.0
Average	2.5	9.1	15.2

increasing temperatures. This suggests greater *in vivo* phagocytosis in resistant chicks due to their higher body temperatures. Significantly, the higher body temperature was most prominent immediately following inoculation when disease organisms are relatively few in number.

Intracellular digestion of bacteria by polymorphonuclear leucocytes

The above experiments suggest greater *in vivo* phagocytosis in resistant chicks because of their higher body temperature. Enhanced phagocytosis could be detrimental if phagocytes were unable to lyse ingested bacteria. In this case, leucocytes would transport bacteria throughout the body. Upon death of leucocytes, liberated bacteria could initiate new foci of infection.

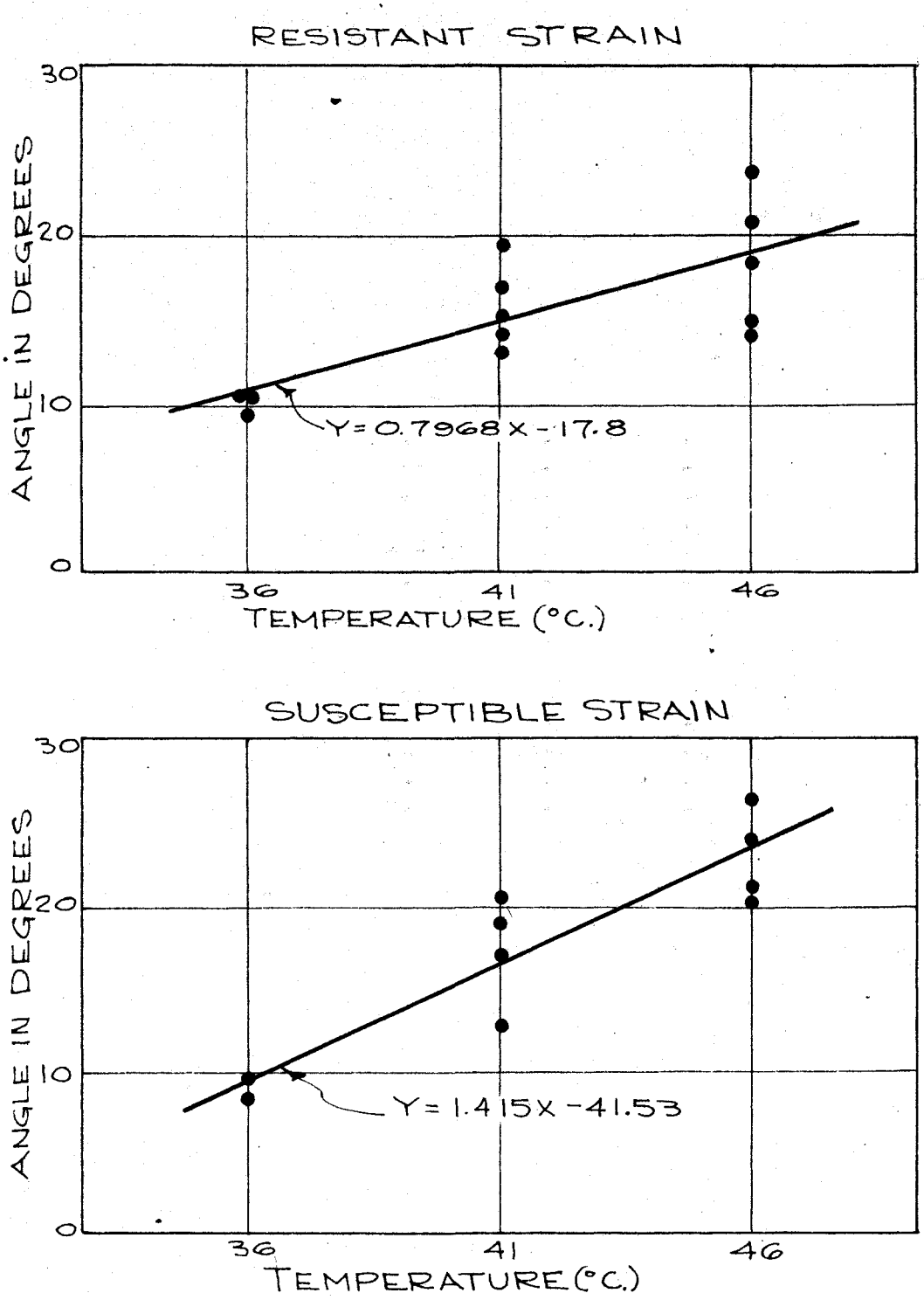


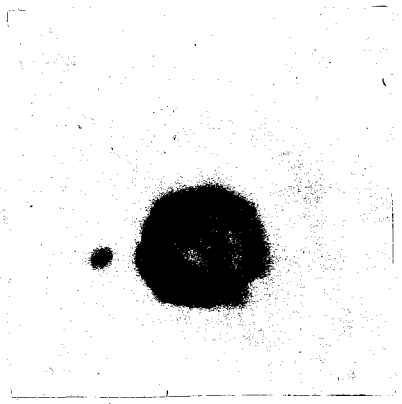
Figure 9. Regressions of angles corresponding to percent phagocytosis on temperature.

The digestive abilities of resistant and susceptible leucocytes were tested in four experiments. Resistant and susceptible strains were equally represented. The phagocytic mixture was agitated for ten minutes at 41°. A sample was removed in the usual manner for determining phagocytosis. Immediately after taking this sample the tubes were replaced in the water bath and left undisturbed for one hour in order that intracellular digestion might occur. After this digestion period another sample was taken from each tube. Digestive ability was based on the difference in bacteria remaining in the sample after one hour digestion contrasted with ingested bacteria found in the first sample. If no digestion occurs, counts made on the two samples should be equal within sampling errors. All slides were stained for the same length of time. The number of polymorphs containing discernible bacteria was determined in the total sample of 400. Photographs of representative polymorphs appear in Plate 1. Bacteria stained bright red while nuclear material appeared as blue. Partially digested bacteria stained lightly and often resembled shadows.

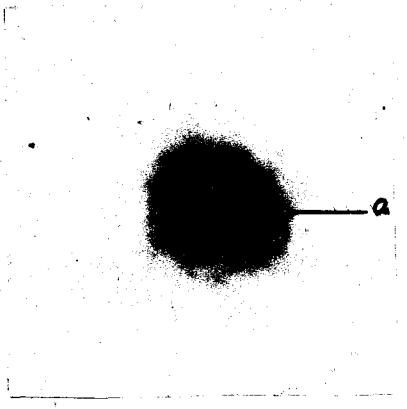
Digestive ability for each individual was estimated from the following ratio:

$$\frac{\text{Number of active polymorphs after digestion}}{\text{Number of active polymorphs before digestion}} \times 100$$

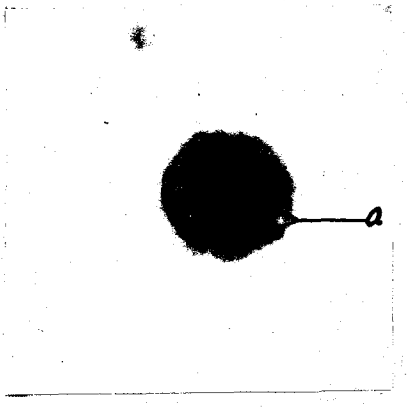
Individuals whose polymorphs lysed a majority of ingested bacteria would have a small digestive ratio. When no digestion occurred a value close to or above 100 would be obtained. This crude estimate of digestive ability has a number of disadvantages. Partially digested bacteria would be considered as not digested. Any bacteria phagocytosed during the digestion period would also bias the results toward no digestion. This type of bias is not serious, since it tends to make the results smaller than the actual



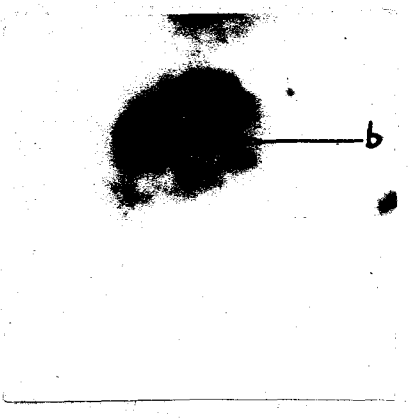
Polymorph with no phagocytosis



Polymorph with phagocytosed bacteria



Polymorph with phagocytosed bacteria



Polymorph showing digestion of phagocytosed bacteria

Plate 1. Polymorphonuclear leucocytes showing phagocytosis and intracellular digestion of bacteria. Note normal bacteria (a) in contrast with light staining and indistinct outline of partially digested bacteria (b). Pappenheim's pyronine-methyl green stain. (X3000).

differences present. Most methods of estimating digestive ability involve the differentiation of partially digested bacteria from normal bacteria. The method used in experiments reported here had the advantage of erasing this personal equation. Even though certain errors are involved, the

Table 20

Intracellular digestion of Shigella gallinarum by polymorphonuclear leucocytes of resistant and susceptible strains

Experiment	Individual digestive ratios	
	Resistant strain	Susceptible strain
I	16.7	83.3
	36.8	69.0
	18.2	78.6
II	46.4	95.2
	45.8	74.0
III	47.1	173.7
IV	26.9	58.0
	45.8	69.2

method used should give an unbiased estimate of differences between resistant and susceptible strains. This is the important question in these studies.

Individual digestive ratios within the resistant and susceptible strains are shown in Table 20. All individuals in the resistant strain had digested more than 50 percent of the phagocytosed bacteria. The susceptible individuals in no case showed 50 percent digestion.

Digestive ratios are percentage data and some approach the upper and lower percent limits. A transformation into angles seemed desirable for a test of significance (Table 21).

The highly significant difference between strains in their intracellular

digestive ability was anticipated from the individual comparisons of Table 21. Under the conditions of these experiments the strains differed markedly in their lytic capacities. Some caution should be used in interpreting

Table 21

Analysis of variance of bacteria digested by resistant and susceptible strains

Source of variation	Degrees of freedom	Mean square
Total	15	
Strains	1	3267.22**
Individuals	14	119.49

** Significant at 1 percent level.

in vitro studies. Even though the leucocytes *in vitro* are still alive, their environment is quite different than in the body. Intracellular digestion *in vitro* can be taken only as an index to *in vivo* activity. Differences between strains as wide as those observed here would be expected to be maintained in the body.

The qualitative difference between individual phagocytes of resistant versus susceptible strains is so pronounced that it could largely account for the differences in resistance observed in Tables 1 and 16.

DISCUSSION

Genetic resistance in fowl typhoid is highly correlated with a high normal body temperature. Resistant strains consistently evidence normal temperatures for 0.2° to 0.4° higher than the susceptible strain. Temperature studies following inoculation indicate that high temperatures alone are insufficient to account for genetic resistance (Tables 7 and 8). Susceptible individuals invariably develop high fever temperatures during the course of the infection. Both between strain and within strain comparisons show fever temperatures in later stages of the disease to be correlated with susceptibility. However, immediately following inoculation resistant chicks possess higher temperatures. Apparently chicks destined to survive are able to maintain higher body temperatures during the incubation period of the disease. This higher temperature could enhance phagocytosis while the pathogens are relatively few in number.

Phagocytic abilities in vitro of polymorphonuclear leucocytes from resistant and susceptible strains are equal at a constant temperature of 41°. Both strains exhibit increased phagocytosis at higher temperature levels. Thus, the higher body temperature of resistant chicks during the disease incubation period suggests a greater phagocytic ability. Other defense mechanisms would probably have increased efficiencies at the more optimum higher temperature. The importance of high body temperature in disease resistance has been considered by other investigators. Scholes and Rutt (1942) concluded it to be a major factor in genetic resistance to Salmonella pullorum in chicks. Temperature differences were also found in Salmonella

pullorum resistant and susceptible strains by Severens et al. (1944), but they assigned the major role in resistance to a larger number of lymphocytes in the resistant strain. These studies are of direct interest here since Salmonella pullorum and Shigella gallinarum infections are similar in many respects. Lambert (1930) observed in the fowl typhoid resistant strains used here a greater resistance to Salmonella pullorum than was evidenced in control strains. This suggests some common defense mechanisms against the two diseases.

To assign major significance to quantitative factors such as higher body temperature or greater number of lymphocytes assumes individual units within defense mechanisms of all strains have equal abilities to destroy or lyse localized bacteria. Studies on the relative intracellular digestive abilities of resistant and susceptible polymorphonuclear leucocytes indicate marked strain differences (Table 21). All resistant individuals lysed more than 50 percent of ingested bacteria after one hour digestion. Susceptible individuals did not evidence such lytic power. This qualitative difference in intracellular digestive enzymes could account for the wide resistance levels to Shigella gallinarum observed in Tables 1 and 16. Other phagocytic cells would have identical genetic constitution as the polymorphs and would probably show similar differences in digestive abilities between strains. Oakberg (1946) reported that the ability of macrophages to digest phagocytosed bacteria increased with increasing genetic resistance to mouse typhoid.

If quantitative differences in defense mechanisms were of major importance in specific disease resistance, they should also impart considerable generalized resistance. Except for closely related diseases, generalized resistance does not appear to be widespread (Gowen, 1948). Qualitative dif-

ferences in intracellular enzymes between resistant and susceptible strains could give resistance to a specific pathogen without imparting resistance to bacteria of different chemical makeup.

In genetic resistance to fowl typhoid high body temperature assumes minor importance when compared with the relative digestive capacities of polymorphonuclear leucocytes from resistant and susceptible strains. Further studies should include lymphocytes as well as wandering and fixed macrophages. These cells could assume equal or greater importance in genetic resistance to Shigella gallinarum.

So far as the author is aware, no investigation of the effect of temperature on intracellular digestion has been reported. Preliminary studies suggest that the rate of in vitro intracellular digestion of Shigella gallinarum varies with the temperature of incubation.

SUMMARY AND CONCLUSIONS

The relationship of body temperature and phagocytic activity of polymorphonuclear leucocytes to genetic resistance was studied in strains of chicks differentiated in their resistance to Shigella gallinarum, the causative organism of fowl typhoid.

Resistant strains of chicks possessed higher normal body temperatures from nine to twenty-three days of age. This higher temperature was also evidenced during the disease incubation period.

Fever temperatures of one to two degrees C. developed in susceptible individuals during the later stages of the disease. Within both resistant and susceptible strains a fever at this stage was associated with susceptibility.

Survivors in both strains evidenced higher temperatures immediately following inoculation than chicks subsequently dying.

Artificially reduced body temperatures lowered resistance in both resistant and susceptible strains. Resistant chicks were more nearly able to maintain normal temperature under adverse conditions.

Phagocytosis in vitro of bacteria by polymorphonuclear leucocytes was equal in resistant and susceptible strains at 41°. Both strains showed greater phagocytosis with increasing temperature. This suggests resistant chicks would have greater phagocytic ability due to higher body temperature.

Little intracellular digestion of phagocytosed bacteria occurred in cells from susceptible strain while marked lysis of bacteria was evidenced by resistant cells. This qualitative difference between phagocytes appears to be an important factor in disease resistance.

Bactericidal properties of whole blood or serum do not appear to be a factor in genetic resistance to Shigella gallinarum.

The ability of polymorphonuclear leucocytes to digest phagocytosed bacteria is a major factor in genetic resistance to fowl typhoid. Differences in body temperature assume an important supporting role.

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